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(57) Abstract

A method of inhibiting polymerization of target proteins by administration of compounds capable of inhibiting aggregation and subsequent transglutaminase-induced cross-linking of adjacent peptides of the target proteins is provided. These compounds are useful as antithrombotic agents and in the treatment of Alzheimer's disease. A method of screening and identifying compounds capable of inhibiting aggregation and subsequent transglutaminase-induced crosslinking of amyloid β peptide is also provided.

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METHOD AND USE OF AGENTS TO INHIBIT PROTEIN POLYMERIZATION AND METHODS OF IDENTIFYING THESE AGENTS

Introduction

5 This invention was made in the course of research supported by the National Institutes of Health. The U.S. Government may have certain rights in the invention.

Background of the Invention

Protein aggregation and the formation of proteinaceous clots or plaques in the
10 body have been implicated in several disease states. Perhaps the most studied proteinaceous polymer is the fibrin clot. Fibrinogen is an inactive protein normally present in the plasma. Thrombin and certain coagulation factors cause fibrinogen to be activated and become fibrin. Fibrin is the substance of blood clots. It is a fibrous protein that forms a polymeric mesh-work over an injured area. The fibrin aggregates
15 become cross-linked by Factor XIII, which is a member of the class of enzymes called transglutaminase. Plasmin, a proteolytic enzyme, gradually dissolves away the clot as tissue repair is taking place. While fibrinogen is an important component in hemostasis, the uncontrolled or abnormal formation of these proteinaceous clots can lead to serious thrombotic and embolic problems, and even death from heart attack or
20 stroke.

Aspirin therapy has been associated with significant clinical antithrombotic effects in certain arterial thromboembolic disorders. It is generally thought that the antithrombotic effect of aspirin is due to O-acetylation by aspirin of active site serine in the platelet enzyme cyclooxygenase. However, aspirin has also been reported to
25 have fibrinolytic and hypoprothrombinemic effects. Several possible mechanisms underlying the enhanced fibrinolysis resulting from aspirin administration have been suggested. Studies designed to investigate these mechanisms were performed by Bjornsson T.D., et al. and reported in *J. Pharmacol. and Exp. Therap.* 1989, 250(1):154. In experiments with ^{14}C acetyl-labeled aspirin and fibrinogen, it was
30 found that fibrinogen is acetylated by aspirin to form ϵ -N-acetyl-lysine on both D and

E domains of the molecule and on the α , β , and γ chains of the molecule. The relationship of this finding to the fibrinolytic effects of aspirin was not understood, however, it was suggested that altered binding resulting from the acetylation or a decrease in the number of Factor XIII-induced cross-links between adjacent fibrin molecules could be responsible. The use of aspirin as an antithrombotic agent is associated with significant gastrointestinal side effects including gastric erosion and occult blood loss.

Amyloid β -peptide is another protein which has been shown to aggregate or polymerize in the body. Deposition in brain tissue of amyloid β -peptide to form neuritic plaques represents an important component of the pathogenesis of Alzheimer's disease. These deposits have been shown to consist in part of insoluble aggregates of the amyloid β -peptide.

It has now been found that polymerization of target proteins such as fibrinogen or amyloid β -peptide is inhibited by contacting the target proteins with a compound capable of inhibiting aggregation and subsequent transglutaminase-induced cross-linking of adjacent peptides of the target proteins. This inhibition of polymerization of target proteins such as fibrinogen and amyloid β -peptide results in the formation of smaller polymers which are less stable. This method and the provided compounds are useful in any situation in which protein aggregation is problematic, i.e., the prevention of blood clotting. These compounds can be used as effective antithrombotic agents or drugs in the treatment of Alzheimer's disease.

Summary of the Invention

An object of the present invention is to provide a method of inhibiting polymerization of target proteins which comprises contacting target proteins with an effective amount of a compound capable of inhibiting aggregation and subsequent transglutaminase-induced cross-linking of adjacent peptides of the target proteins.

Another object of the present invention is to provide uses for these compounds including use as antithrombotic drugs, drugs in the treatment of Alzheimer's disease, and other diseases caused by amyloidosis or protein polymerization.

Another object of the present invention is to provide a method of screening and identifying compounds capable of inhibiting aggregation and subsequent transglutaminase-induced crosslinking of adjacent peptides.

Brief Description of the Figures

Figure 1 is a bar graph showing the percent inhibition of transglutaminase-induced cross-linking of amyloid β -peptide following preincubation with compounds

1 through 8 and compounds S1 through S3 for 6 hours. Aspirin (ASA) was also preincubated for comparison.

Figure 2 is a bar graph showing the thrombin times following incubation with a compounds 1 through 8 for 10 minutes and 6 hours and compounds S1 through S3 for 10 minutes and 1.5 hours. Aspirin (ASA) was also incubated for comparison.

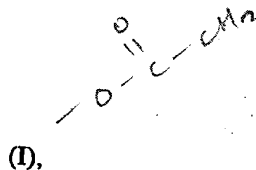
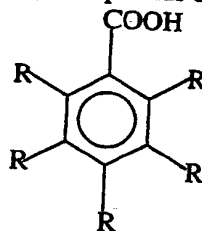
Detailed Description of the Invention

Transglutaminase-induced cross-linking forms cross-links between lysyl and glutamyl residues on adjacent peptides. More specifically, transglutaminases catalyze Ca^{2+} -dependent acyl transfer reactions between γ -carboxamide groups of peptide-bound glutamine residues, which serve as acyl donors, and various primary amines, resulting in new γ -amides of glutamic acid and ammonia. The most common acyl acceptor substrates involve ϵ -amino groups of peptide-bound lysyl residues and the primary amino groups of polyamines. The involvement of lysyl residues results in the formation of ϵ -(γ -glutamine)lysine cross-links, which are found in a variety of tissues. An example of such cross-links is the Factor XIII-induced cross-link formation of fibrin clots in the blood. Transglutaminases are present in various tissues, cells, and fluids, including endothelial and glial cells, hepatocytes, and blood.

It has now been found that inhibition of transglutaminase-induced cross-linking inhibits polymerization of many proteins. Inhibition of this cross-linking can be achieved by acetylation of lysyl residues of adjacent peptides which form these cross-links. Inhibition of the cross-linking ultimately results in inhibition of polymerization, formation of smaller polymers and, in the case of fibrin clots, more readily digestable polymers. In the present invention, a method is provided for inhibiting polymerization of target proteins which comprises contacting target proteins with an effective amount of a compound capable of inhibiting transglutaminase-induced cross linking of adjacent peptides of the target proteins. Methods are also provided in the present invention for screening and identifying compounds capable of inhibiting transglutaminase-induced cross linking of adjacent peptides. For the purposes of this invention the term "target protein" refers to any protein which can undergo aggregation and subsequent transglutaminase-induced cross-linking polymerization. Examples of target proteins include, but are not limited to, fibrinogen and amyloid β -peptide. In experiments wherein synthetic amyloid β -peptide ($\text{A}\beta_{1-40}$) was incubated with transglutaminase, synthetic $\text{A}\beta_{1-40}$ formed dimers (A_2), tetramers (A_4), pentamers (A_5) and hexamers (A_6). Inhibition of transglutaminase-induced cross-linking of target proteins by acetylation of the adjacent peptides of the target proteins, results in decreased transglutaminase-induced cross-linking. The term "effective amount" refers to a concentration of a compound which results in a

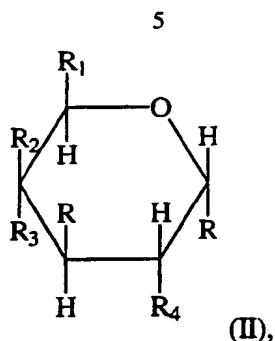
measurable interference with the aggregation and subsequent cross-linking of adjacent peptides of target proteins. Such concentrations can be easily determined by one of skill in the art in accordance with the teachings of the present invention. In the case of fibrinogen, inhibition of aggregation and polymerization results in the prolongation of thrombin time and then in the formation of fibrin clots that are less cross-linked by the circulating transglutaminase Factor XIII and are more readily lyzed by the proteolytic enzyme plasmin. Such treatment allows for the formation of physiological stable clots, e.g., for normal clot formation, while making abnormal clots easier to lyse. This treatment provides a safer antithrombotic agent with less risk of bleeding. In addition, the compounds provided in this disclosure do not have the adverse side effects associated with other antithrombotic agents such as aspirin. In the case of amyloid β -peptide, a peptide that forms neuritic plaques on the brains of patients with Alzheimer's disease, inhibition of transglutaminase-induced cross-linking results in smaller polymers and reduced plaque formation.

It has now been found that compounds containing at least one acetyl group, when placed in contact with target proteins, significantly altering the polymerization and function of the target proteins. Examples of compounds containing at least one acetyl group which can be used in the present invention include, but are not limited to, a variety of simple benzoic acid derivatives having at least one acetyl group, simple sugars, sugar acids, and amino sugars and their derivatives, having at least one acetyl group and amino acid derivatives having at least one acetyl group. Simple benzoic acids are exemplified by compounds of Formula (I):



wherein

R is selected from a group consisting of H, OH and OCOCH_3 . Simple sugars, sugar acids, and amino sugars and their derivatives are exemplified by compounds of Formula (II):



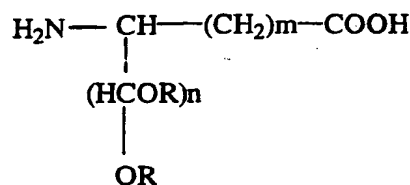
wherein

R is selected from a group consisting of OH and OCOCH₃;

R₁ is selected from a group consisting of COOH and CH₂OCOCH₃;

5 R₂ and R₃ are selected from a group consisting of H, OH and OCOCH₃; and

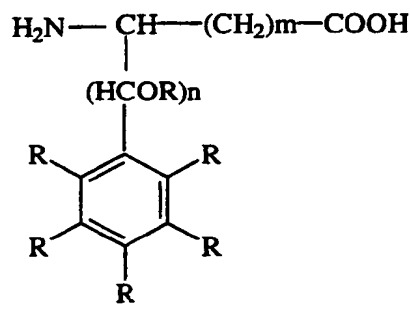
R₄ is selected from a group consisting of OH, OCOCH₃, NH₂ and NHCOCH₃, with the proviso that R₂ and R₃ are different and one of R₂ or R₃ is H. Amino acid derivatives having at least one acetyl group are exemplified by compounds of Formula (III):



10

(III), and

Formula (IV):



(IV),

wherein

15 m is a number from 0 to 10;

n is a number from 0 to 10; and

R is selected from a group consisting of H, OH or OCOCH₃.

Compounds or agents containing at least one acetyl group can be used in any application where formation of proteinaceous clots is problematic, i.e. for prevention of blood clotting. In addition the compounds can be used therapeutically to inhibit polymerization of target proteins such as fibrinogen and amyloid β-peptide. For example, compounds in the present invention can be used as antithrombotic agents or

20

as drugs in the treatment of Alzheimer's disease. A pharmaceutical composition useful in the present invention comprises a compound capable of inhibiting aggregation and subsequent transglutaminase-induced cross-linking and an appropriate carrier, diluent, or excipient. Such pharmaceutical carriers, diluents, or excipients may be either solid or liquid. Such pharmaceutical compositions may be parenterally, rectally, topically, transdermally or orally administered, preferably orally. Pharmaceutical forms include, but are not limited to, tablets, capsules and lozenges, or syrups, suspensions, and emulsions.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier routinely used for solid formulation. Examples of such carriers include, but are not limited to, magnesium stearate, starch, lactose, sucrose and cellulose. A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets, granules or powder containing a compound capable of inhibiting aggregation and subsequent transglutaminase-induced cross-linking can be prepared using standard carriers and then filled into a hard gelatin capsule. Alternatively, a dispersion or suspension can be prepared using a suitable pharmaceutical carrier and the dispersion or suspension is then filled into a soft gelatin capsule. Suitable pharmaceutical carriers include, but are not limited to, gums, celluloses, silicates and oils.

A liquid formulation will generally consist of a suspension or solution of the compound in a suitable liquid carrier. Suitable liquid carriers include, but are not limited to, ethanol, glycerin, non-aqueous solvents such as polyethylene glycol, oils, or water with a suspending agent, preservatives, flavorings, or coloring agents or any suitable combination thereof.

A composition for parenteral administration can be formulated as solution or a suspension. Said solution or suspension will generally consist of a compound capable of inhibiting aggregation and subsequent transglutaminase-induced cross-linking in a sterile aqueous carrier or parenterally acceptable oil. Examples of parenterally acceptable oils include, but are not limited to, polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oils and sesame oils. Alternatively, the solution can be lyophilized and then reconstituted with a suitable solvent just prior to administration.

The pharmaceutical preparations are made following convention techniques of a pharmaceutical chemist and involve mixing, granulating, and compressing, when necessary for tablet forms, or mixing, filling and dissolving the ingredients, as appropriate, to give the desired oral, parenteral, rectal, transdermal, or topical products.

Preferably the composition is in unit dose form. Doses can be easily ascertained by one of skill in the art in accordance with a patient's age, weight and other determined parameters.

These compounds and others were identified as capable of inhibiting transglutaminase-induced cross-linking of peptides with the screening method of the present invention. The present invention provides a method by which to quantitate pharmacologic inhibition of transglutaminase-induced cross-linking of amyloid β peptide. In this method, potential inhibitors of the transglutaminase-induced cross-linking of amyloid β peptide are co-incubated with synthetic 40-amino acid human amyloid β peptide ($A\beta_{1-40}$) and guinea pig liver transglutaminase. After the incubation, a mixture of sodium dodecyl sulfate (SDS), urea, glycerol and β -mercaptoethanol is added to each sample and incubated for several hours. The samples are then boiled and loaded onto individual lanes of an SDS polyacrylamide gel, separated, stained with Coomassie blue and the resultant protein bands quantitated. The percent inhibition of transglutaminase-induced cross-linking is determined by correcting for the amount of non-cross-linked $A\beta_{1-40}$ in control samples incubated without any test compounds. Initially, the densitometric values for the nonpolymerized monomer (A_1), and for the cross-linked dimer (A_2), and the polymers, tetramer (A_4), pentamer (A_5), and hexamer (A_6), were normalized to fractions of unity assigned to the sum of the readings for the individualized bands. Subsequently, the percent inhibition was derived using the normalized values for the control and treated samples; the latter are identified by an asterisk. Percent inhibition is determined by:

$$\text{Percent Inhibition (\%)} = [1 - (A_n/A_n^*)] \times 100$$

where A_n and A_n^* are the sums of normalized polymer bands (dimer, tetramer, pentamer, hexamer or any higher detectable polymers) in treated and nontreated samples, respectively. Concentration versus percent inhibition curves after pharmacologic inhibition of transglutaminase-induced cross-linking of amyloid β peptide can then be constructed. This method has the advantage that potential variation in the total amounts of amyloid polymers added to each lane on the gel do not affect the analysis, since the distribution of individual band intensities within each lane is normalized to unity. This allows the subtraction of the amount of the nonpolymerized amyloid β peptide in a control sample from that in a treated sample. This method provides a meaningful comparison of the inhibitory potential across different pharmacologic inhibitors of transglutaminase-induced amyloid β peptide

cross-linking. A number of compounds containing at least one acetyl group were screened using the method of the present invention. In addition, a number of compounds including known transglutaminase inhibitors such as dansylcadaverine and spermine, and agents reported to have some therapeutic activity in Alzheimer's disease or compounds related to such agents such as the non-steroidal anti-inflammatory drugs indomethacin, diflunisal, and meclofenamic acid, the monamine oxidase inhibitors tranylcypromine and phenelzine, the iron chelating agent deferoxamine, and the acetylcholinesterase inhibitor tacrine were shown to inhibit transglutaminase-induced β peptide cross-linking with this method.

To further illustrate the present invention, the following nonlimiting examples are provided:

EXAMPLES

Example 1: Transglutaminase concentration dependency

Synthetic A β ₁₋₄₀ (0.33 mg/ml) was incubated at 22°C with guinea pig liver transglutaminase (0, 2.5, 5.0, 25, 50, and 250 μ g/ml) in 50 mM Tris-HCl, pH 7.4, containing 25 mM CaCl₂ and 15 mM dithiothreitol. All concentrations are expressed as final concentrations in a total volume of 75 μ l. Sample were incubated for 4 hours. After incubation, a 75 μ l mixture of sodium dodecyl sulfate (SDS 4%), urea (2 M), and mercaptoethanol (5%) was added to the sample, mixed and incubated at 37°C for 10 hours. Following this second incubation, the samples were boiled and aliquots (40 μ l) were loaded on individual lanes on 10% SDS polyacrylamide gels with 2.5% SDS stacking gels, separated, stained with Coomassie blue, and quantitated using a laser scanning densitometer. Similar polymerization was observed at 2.5 mM, 5 mM and 25 mM CaCl₂. To investigate pH-dependence, the effects of three different pHs (pH 6.4, 7.4 and 8.4) during the incubation with transglutaminase were evaluated in separate experiments; A β polymer formation at pH 8.4 was similar to that at pH 7.4, however, at pH 6.4 polymer formation was decreased. To investigate Ca⁺²-dependence, edetate disodium (EDTA) was added in equal molar ratio with Ca⁺², and Zn⁺² was added in various concentrations to generate different molar ratios with Ca⁺². Both EDTA and Zn⁺² initiated polymer formation in the presence of Ca⁺². Maximal polymer formation was observed with 50 μ g/ml transglutaminase.

Example 2: Time course experiments

Synthetic A β ₁₋₄₀ (0.33 mg/ml) was incubated at 22°C with guinea pig liver transglutaminase (50 μ g/ml) in 50 mM Tris-HCl, pH 7.4, containing 25 mM CaCl₂ and 15 mM dithiothreitol. Incubation times were varied from 5 minutes to 24 hours.

Polymer formation was determined as described in Example 1. Maximum polymer formation was observed after 50 µg/ml transglutaminase and after 4 hours incubation.

Example 3: Human Factor XIII incubations

- 5 In separate experiments, transglutaminase was replaced by human Factor XIII (50 and 250 µg/ml) which had been pretreated with bovine thrombin (10 NIH U/ml) in the presence of CaCl₂ (5 mM) at 37°C for 1 hour. Incubation with activated Factor XIII only resulted in limited A₂ formation .

10 Example 4: Effects of compounds on amyloid β-peptide as measured by percent inhibition of transglutaminase-induced cross-linking

- Compounds 1 through 8 and S1 through S3 (shown in Table 1 following) were dissolved in 0.15 M NaCl, 0.01 M sodium citrate, pH 7.4, to produce standard solutions of 50 mM and were incubated with synthetic amyloid β-peptide (0.33 mg/ml) in final concentration of 2.5, 5.0 and 10 mM at 22°C for 6 hours. The amyloid β-peptide containing samples were then incubated at 22°C with guinea pig liver transglutaminase (50 µg/ml) in 50 mM Tris-HCl, pH 7.4, containing 25 mM CaCl₂ and 15 mM dithiothreitol, in a total volume of 75 µl. A number of different pharmacologic agents known to either inhibit transglutaminase or have some therapeutic activity in Alzheimer's disease or compounds related to such agents were also co-incubated with these reactants under the same conditions. These were the transglutaminase inhibitors dansylcadaverine (0.0001 to 2 mM) and spermine (1 and 10 mM), the non-steroidal anti-inflammatory agents indomethacin (0.25 to 4 mM), diflunisal (1 and 10 mM), meclofenamic acid (0.5 to 5 mM), and salicylic acid (1 and 10 mM), the monamine oxidase inhibitors tranylcypromine (0.25 to 10 mM) and phenelzine (1 and 10 mM), the iron chelating agent deferoxamine (1 to 10 mM), and the acetylcholinesterase inhibitor tacrine (1 to 20 mM). After the incubation, a 75 µl mixture of sodium dodecyl sulfate (SDS; 4%), urea (2M), and mercaptoethanol (5%) was added, mixed and incubated at 37°C for 10 hours. After boiling, 40 µl aliquots were loaded onto SDS polyacrylamide gels as described in Example 1 and polymer formation was determined.

- In the untreated samples, in addition to the monomer band, there were cross-linked polymer bands of the amyloid β-peptide (dimers, tetramers, pentamers, hexamers or any higher detectable polymers). Percent inhibition was computed using the expression:

$$\text{Percent Inhibition (\%)} = [1 - (A_n/A_n^*)] \times 100$$

where A_n and A_n^* are the sums of normalized polymer bands (dimers, tetramers, pentamers, hexamers or any higher detectable polymers) in treated and nontreated samples, respectively.

5 Figure 1 shows the percent inhibition of transglutaminase-induced cross-linking of amyloid β -peptide following preincubation with compounds 1 through 8 and S1 through S3. All compounds had inhibiting effects on transglutaminase-induced cross-linking of the β -peptide, with the exception of compound 5. The four most potent compounds were 2, 6, 7 and 8. Of the known pharmacologic agents
10 tested, the most potent were the standard transglutaminase inhibitor dansylcadaverine, with an IC_{50} of 40.9 μ M, and monamine oxidase inhibitor tranylpromine, with an IC_{50} of 100.8 μ M. Other agents were found to have considerable inhibitory activity, with approximate 50% inhibition between 1 and 10 mM, i.e., indomethacin, meclofenamic acid, diflunisal, phenelzine, spermine and deferoxamine. The
15 acetylcholinesterase inhibitor tacrine had an IC_{50} of 67.31 mM. Salicylic acid was ineffective in this assay.

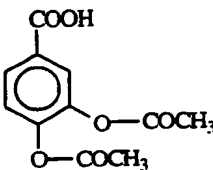
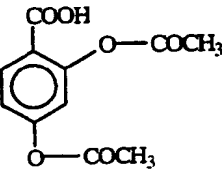
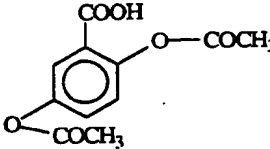
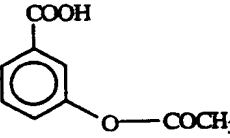
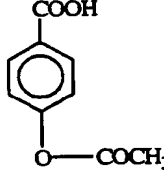
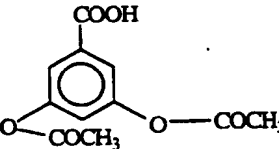
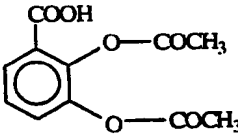
Example 5: Effects of compounds containing at least one acetyl group on fibrinogen as measured by thrombin time

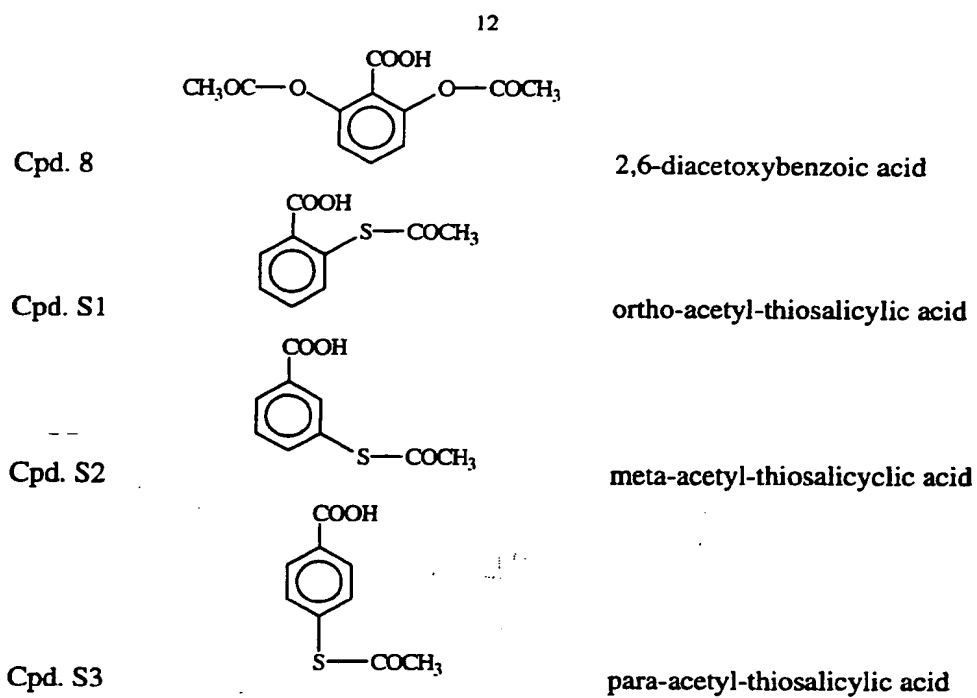
20 The compounds in Table 1 following were dissolved as described in Example 4. They were then added to a human fibrinogen solution (1.5 mg/ml in 0.15 M NaCl, 0.01 M sodium citrate, pH 7.4) to yield a final concentration of 5 mM. The samples containing compounds 1 through 8 were incubated at 37°C for 10 minutes and 6 hours. The samples containing compounds S1 through S3 were incubated at 37°C for
25 10 minutes and 1.5 hours. After incubation thrombin times were determined. The thrombin time determinations were performed using standard methods, involving mixing, and then incubating 100 μ l of the fibrinogen solution (with the different compounds) and 100 μ l of imidazole-buffered saline (0.15 NaCl, 0.045 M imidazole, pH 7.4) at 37°C for 5 minutes. After incubation 100 μ l of bovine thrombin solution
30 (10 NIH units in 0.15 NaCl, 0.01 M sodium citrate, pH 7.4) was added to initiate the coagulation process. The thrombin time was measured with a fibrometer coagulation timer. Each compound in Table 1 was tested in duplicate and the results averaged.

 Figure 2 shows the thrombin times for compounds 1 through 8 and compounds S1 through S3. Compounds S1 through S3 all caused thrombin times >200 seconds
35 following a 6 hour incubation. Following a 1.5 hour incubation compounds S2 and S3 caused thrombin times >200, however, compound S1 caused thrombin time of 92.8 sec. The six most potent compounds were compounds 1, 2, 6 and 7 and compounds

S2 and S3. All compounds caused some prolongation in thrombin times after only 10 minute incubations.

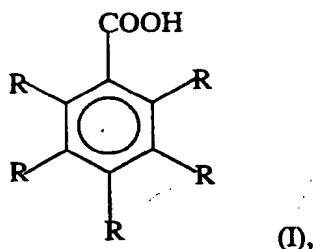
Table 1: Compounds of Examples 4 and 5

5	Compound Number	Chemical Structure	Chemical Name
	Cpd. 1		3,4-diacetoxybenzoic acid
	Cpd.2		2,4-diacetoxybenzoic acid
	Cpd. 3		2,5-diacetoxybenzoic acid
	Cpd. 4		meta-acetoxybenzoic acid
10	Cpd. 5		LR67 para-acetoxybenzoic acid
	Cpd. 6		3,5-diacetoxybenzoic acid
	Cpd. 7		2,3-diacetoxybenzoic acid

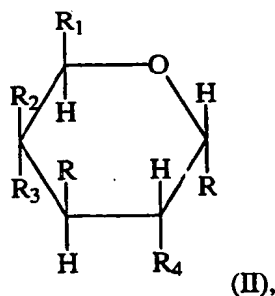


What is claimed is:

1. A method of inhibiting polymerization of target proteins comprising contacting target proteins with an effective amount of a compound capable of inhibiting aggregation and subsequent transglutaminase-induced cross-linking of adjacent peptides of the target proteins.
2. The method of claim 1 wherein the compound contains at least one acetyl group.
3. The method of claim 2 wherein the compound is selected from a group consisting of Formula (I):



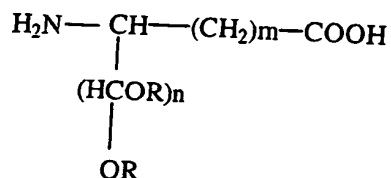
- wherein
R is selected from a group consisting of H, OH and OCOCH₃.

Formula (II):

- wherein
R is selected from a group consisting of OH and OCOCH₃;
R₁ is selected from a group consisting of COOH and CH₂OCOCH₃;
R₂ and R₃ are selected from a group consisting of H, OH and OCOCH₃; and
R₄ is selected from a group consisting of OH, OCOCH₃, NH₂ and NHCOCH₃, with the proviso that R₂ and R₃ are different and one of R₂ or R₃ is H;

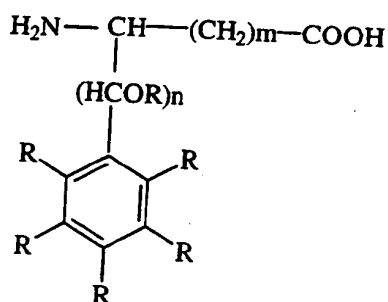
Formula (III):

14



(III), and

Formula (IV):



(IV),

wherein

m is a number from 0 to 10;

n is a number from 0 to 10; and

R is selected from a group consisting of H, OH or OCOCH₃.

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4. The use of compounds containing at least one acetyl group as antithrombotic agents.

5. The use of compounds containing at least one acetyl group in the treatment of Alzheimer's disease.

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6. A method of screening and identifying compounds capable of inhibiting aggregation and subsequent transglutaminase-induced crosslinking of amyloid β peptide comprising:

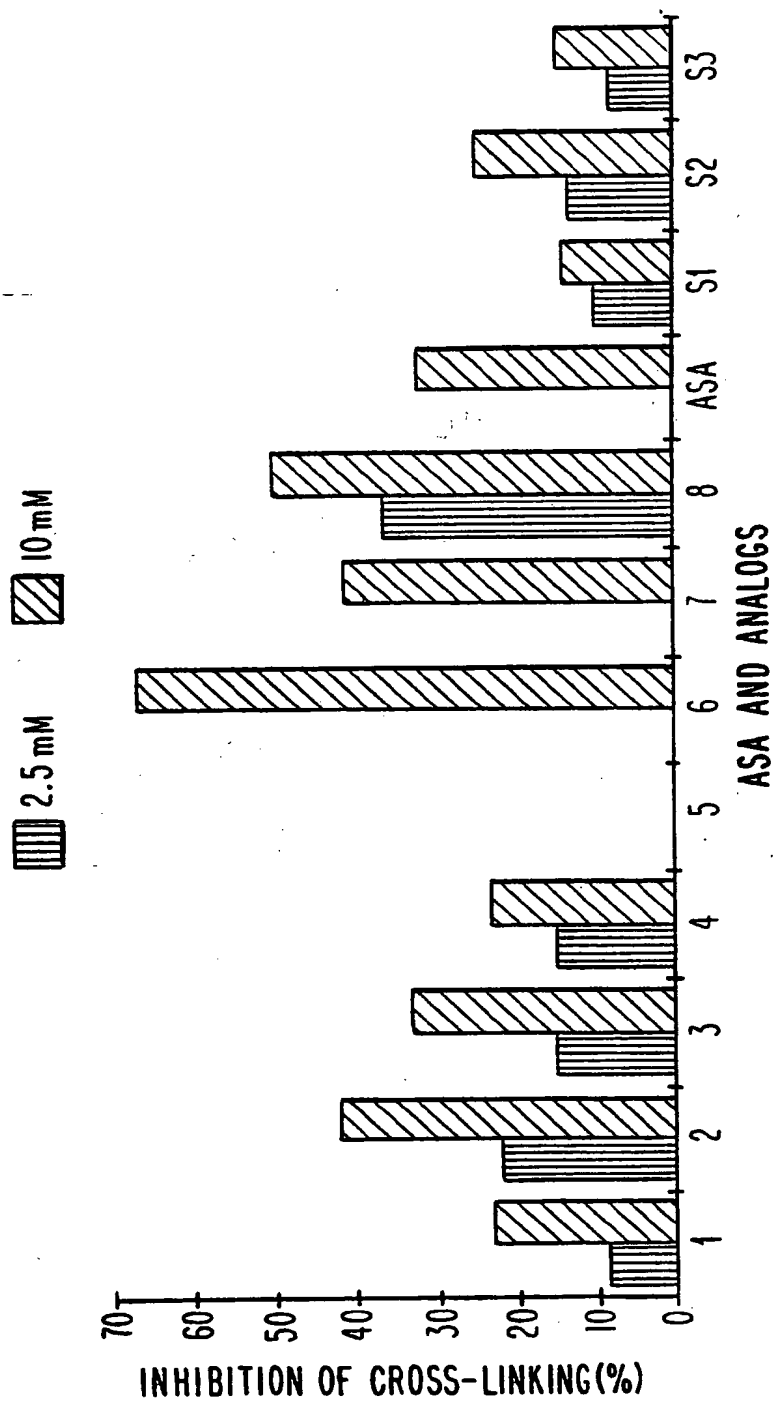
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- (a) incubating a compound with A β ₁₋₄₀ and a transglutaminase;
- (b) separating any amyloid β polymers formed during the incubation by gel electrophoresis;
- (c) quantifying protein bands on the gel; and
- (d) determining percent inhibition of transglutaminase-induced cross-

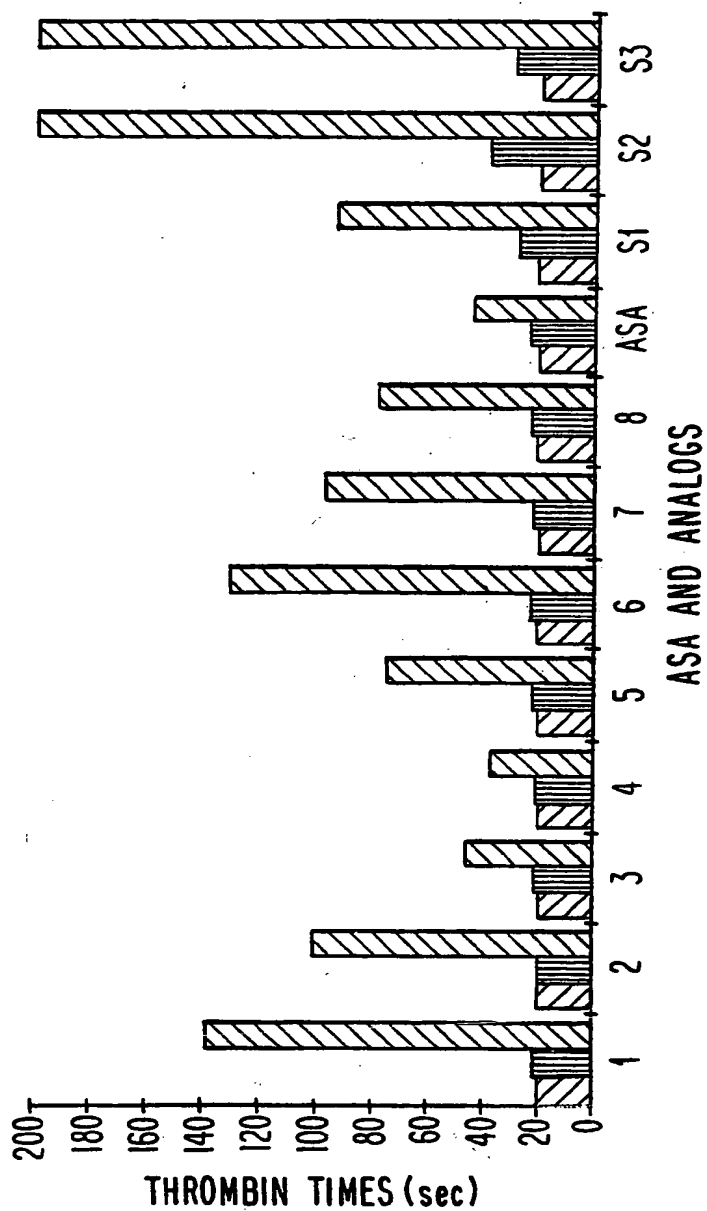
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linking of amyloid β peptide.

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**Fig. 1****SUBSTITUTE SHEET (RULE 26)**

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**Fig. 2****SUBSTITUTE SHEET (RULE 26)**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06383

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/11, 31/60.

US CL :514/165, 699.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/165, 699.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG:

TERMS SEARCHED: FORMULAE OF CLAIM 3 AND ALZHEIMER'S AND POLYMERIZATION AND PROTEINS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US, A, 5,192,753 (McGEER ET AL) 09 March 1993, SEE ENTIRE DOCUMENT.	1-2 ----- 3-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 AUGUST 1995

Date of mailing of the international search report

13 SEP 1995

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